University of the Highlands and Islands
Oilthigh na Gàidhealtachd agus nan Eilean

UHI Research Database pdf download summary

Insights into the red algae and eukaryotic evolution from the genome of Porphyra umbilicalis (Bangiophyceae, Rhodophyta)

Brawley, Susan H.; Blouin, Nicolas A.; Ficko-blean, Elizabeth; Wheeler, Glen L.; Lohr, Martin; Goodson, Holly V.; Jenkins, Jerry W.; Blaby-haas, Crysten E.; Helliwell, Katherine E.; Chan, Cheong Xin; Marriage, Tara N.; Bhattacharya, Debashish; Klein, Anita S.; Badis, Yacine; Brodie, Juliet; Cao, Yuanyu; Collén, Jonas; Dittami, Simon M.; Gachon, Claire M. M.; Green, Beverley R.

Published in:
Proceedings of the National Academy of Sciences of the United States of America

Publication date:
2017

Publisher rights:
Copyright © 2017 National Academy of Sciences

The Document Version you have downloaded here is:
Peer reviewed version

The final published version is available direct from the publisher website at:
10.1073/pnas.1703088114

Link to author version on UHI Research Database

Citation for published version (APA):
Brawley, S. H., Blouin, N. A., Ficko-blean, E., Wheeler, G. L., Lohr, M., Goodson, H. V., Jenkins, J. W., Blaby-haas, C. E., Helliwell, K. E., Chan, C. X., Marriage, T. N., Bhattacharya, D., Klein, A. S., Badis, Y., Brodie, J., Cao, Y., Collén, J., Dittami, S. M., Gachon, C. M. M., ... Prochnik, S. E. (2017). Insights into the red algae and eukaryotic evolution from the genome of Porphyra umbilicalis (Bangiophyceae, Rhodophyta). *Proceedings of the National Academy of Sciences of the United States of America*, *14*(31), 6361-6370. https://doi.org/10.1073/pnas.1703088114

General rights
Copyright and moral rights for the publications made accessible in the UHI Research Database are retained by the authors and/or other copyright owners and it is a condition of accessing publications that users recognise and abide by the legal requirements associated with these rights:

1) Users may download and print one copy of any publication from the UHI Research Database for the purpose of private study or research.
2) You may not further distribute the material or use it for any profit-making activity or commercial gain
3) You may freely distribute the URL identifying the publication in the UHI Research Database

Take down policy
If you believe that this document breaches copyright please contact us at RO@uhi.ac.uk providing details; we will remove access to the work immediately and investigate your claim.

Download date: 12. Sep. 2025

Thriving in a tough environment: Insights into the red algae from the genome of *Porphyra umbilicalis* (Bangiophyceae, Rhodophyta).

Short Title: The genome of the red alga *Porphyra umbilicalis*

Classification: Biological Sciences (Major); Evolution (Minor)

Susan H. Brawley[a], Nicolas A. Blouin[a,b], Elizabeth Ficko-Blean[c], Glen L. Wheeler[d], Martin Lohr[e], Holly V. Goodson[f], Jerry W. Jenkins[g,u], Crysten E. Blaby-Haas[h], Katherine E. Helliwell[d,i], Cheong Xin Chan[j], Tara Marriage[k], Debashish Bhattacharya[l], Anita S. Klein[m], Yacine Badis[n], Juliet Brodie[o], Yuanyu Cao[m], Jonas Collén[c], Simon M. Dittami[c], Claire M. M. Gachon[n], Beverley R. Green[p], Steven Karpowicz[q], Jay W. Kim[r], Ulrich Johan Kudahl[i], Senjie Lin[s], Gurvan Michel[c], Maria Mittag[t], Bradley J. S. C. Olson[k], Jasmyn Pangilinan[u], Yi Peng[u], Huan Qiu[l], Shengqiang Shu[u], John T. Singer[v], Alison G. Smith[i], Brittany N. Sprecher[s], Volker Wagner[t], Wenfei Wang[w], Zhi-Yong Wang[x], Juying Yan[u], Charles Yarish[y], Simone Zäuner-Riek[z], Yun Yun Zhuang[s], Yong Zou[t], Erika A. Lindquist[u], Jane Grimwood[g,u], Kerrie Barry[u], Daniel S. Rokhsar[u], Jeremy Schmutz[g,u], John W. Stiller[aa], Arthur R. Grossman[w], Simon E. Prochnik[u,*]

[a]School of Marine Sciences, University of Maine, Orono, ME 04469; [b]Dept of Molecular Biology, University of Wyoming, Laramie, WY 82071; [c] Sorbonne Universités, UPMC Univ Paris 06, CNRS, UMR 8227, Integrative Biology of Marine Models, Station Biologique de Roscoff, CS 90074, 29688 Roscoff, France; [d]Marine Biological Association of the United Kingdom, The Laboratory, Citadel Hill, Plymouth, PL1 2PB, UK; [e] Johannes Gutenberg-Universität Mainz, Institut für Molekulare Physiologie, Pflanzenbiochemie, Johannes-von-Müller-Weg 6, 55128 Mainz, Germany; [f]Dept. of Chemistry & Biochemistry, University of Notre Dame, South Bend, IN 46556, USA; [g]Hudson-Alpha Institute for Biotechnology, 601 Genome Way Northwest, Huntsville, AL 35806 USA; [h]Biology Dept, Brookhaven National Laboratory, Brookhaven Ave, Upton, NY 11973 USA; [i]Dept. of Plant Sciences, University of Cambridge, Downing St, Cambridge CB2 3EA, UK; [j]Institute for Molecular Bioscience, and School of Chemistry and Molecular Biosciences, University of Queensland, Brisbane QLD 4072,

Australia; [k]Division of Biology, Kansas State University, Manhatten, KS 66506, USA; [l]Dept Ecology, Evolution & Natural Resources, Rutgers University, New Brunswick, NJ 08901, USA; [m]Dept of Biological Sciences, University of New Hampshire, Durham, NH 03824; [n]Scottish Association for Marine Sciences, Scottish Marine Institute, PA37 1QA, Oban, UK; [o]Division of Life Sciences, The Natural History Museum, Cromwell Road, London SW7 5BD, UK; [p]Dept. of Botany, 3200-6270 University Blvd, University of British Columbia, Vancouver BC, V6T 1Z4, Canada; [q]Tau Biosciences LLC, Edmond, 73003 OK; [r]Dept of Biomolecular Engineering, University of California Santa Cruz, Santa Cruz, CA, USA; [s]Dept of Marine Sciences, University of Connecticut, 1080 Shennecossett Road, Groton, CT 06340, USA(current address, YYZ: College of Environmental Science and Engineering, Ocean University of China, 238 Songling Road, Qingdao 266100, China); [t]Institut für Allgemeine Botanik und Pflanzenphysiologie, Friedrich-Schiller-Universität Jena, Am Planetarium 1, 07743 Jena, Germany; [u]LBNL Joint Genome Institute, 2800 Mitchell Dr, Walnut Creek, CA 94598; [v]Dept of Molecular & Biomedical Science, University of Maine, Orono, ME 04469; [w] Basic Forestry and Proteomics Research Center, HIST, Fujian Agriculture and Forestry University, Fuzhou 350002, China; [x]Dept. of Plant Science, Carnegie Institution for Science, Stanford, CA; [y]Dept of Ecology & Evolutionary Biology, University of Connecticut, Stamford, CT 06901, USA; [z]Institute of Molecular Physiology and Biotechnology, University of Bonn, Karlrobert-Kreiten-Str. 13, 53115 Bonn, Germany; [aa]Dept of Biology, East Carolina University, Greenville, NC 27858, USA

Communicating Author:
Susan H. Brawley
School of Marine Sciences, University of Maine
Orono, ME 04469
207-581-2973; brawley@maine.edu

*Authors' Contributions:
Designed research: SHB, JWS, ARG, NAB, DB, SL, KB, CY, DSR, JS, SEP
Isolated/ grew cultures and/or prepared/screened nucleic acid samples: SHB, NAB, JB, SL, JTS, CY, YYZ, JG, ARG; Contributed analytical tools: SHB, NAB, EF-B, GLW, ML, HVG, JWJ, KEH, CXC, DB, SMD, CG, SK, JK, GM, JP, YP, HQ, SQS, AGS, JY, EAL, JG, SEP;

Analyzed data: SHB, NAB, EF-B, GLW, ML, HVG, JWJ, CEB-H, KEH, CXC, TM, DB, ASK, YB, YC, JC, SMD, CMMG, BRG, SK, JK, UJK, SL, GM, MM, BJSCO, HQ, AGS, BS, VW, WW, ZYW, SZ-R, YYZ, YZ, JS, JWS, ARG, SEP; Wrote the paper: SHB, NAB, EF-B, GLW, ML, HVG, CEB-H, KEH, TM, DB, ASK, YB, JB, YC, JC, SMD, CMMG, BRG, SK, SL, MM, BJSCO, HQ, AGS, BS, WW, ZYW, CY, SZ-R, JWS, ARG, SEP

SIGNIFICANCE STATEMENT

Fossil evidence shows that red algae (Rhodophyta) are one of the most ancient multicellular lineages. Their ecological, evolutionary, and commercial importance notwithstanding, few red algal nuclear genomes have been sequenced. Our analysis of the *Porphyra umbilicalis* genome provides insight into how this macrophyte thrives in the stressful intertidal zone and to the basis for its nutritional value as human food. Many of the novel traits (e.g., cytoskeletal organization, calcium signaling pathways) we find encoded in the *Porphyra* genome are extended to other red algal genomes, and our unexpected findings offer a potential explanation for why the red algae are constrained to small stature and cell size relative to other multicellular lineages.

ABSTRACT

Porphyra umbilicalis (laver) belongs to an ancient group of red algae (Bangiophyceae), is harvested for human food, and thrives in the harsh conditions of the upper intertidal zone. Here we present the 87.7 Mbp haploid *Porphyra* genome (65.8% G + C-content, 13,125 gene loci) and elucidate traits that inform our understanding of the biology of red algae as one of the few multicellular eukaryotic lineages. Novel features of the *Porphyra* genome that are shared by other red algae relate to the cytoskeleton, calcium signaling, the cell cycle, and stress tolerance mechanisms including photoprotection. Cytoskeletal motor proteins in *Porphyra* are restricted to a small set of kinesins that appear to be the only universal cytoskeletal motors within the red algae. Dynein motors are absent, and *Porphyra* and most other red algae lack myosin. This surprisingly minimal cytoskeleton offers a potential explanation for why red algal cells and individual organisms are smaller than those of other multicellular lineages. Additional discoveries further relating to the stress tolerance of bangiophytes include ancestral enzymes for sulfation of the hydrophilic galactan-rich cell wall, evidence for mannan synthesis that originated before the divergence of green and red algae, and a high capacity for nutrient uptake. Our analyses provide a comprehensive understanding of the red algae, which are both commercially important and have played a major role in the evolution of other algal groups through secondary endosymbioses.

Keywords: antioxidants, calcium signaling, carbohydrate-active enzymes (CAZymes), cyclin D, dynein, iron-uptake, kinesin, mannans, mycosporine-like amino acids (MAAs), myosin, porphyran, sulfated galactans, vitamin B_{12}

Introduction The red algae are one of the founding groups of photosynthetic eukaryotes (Archaeplastida) and among the few multicellular lineages within Eukarya. A red algal plastid, acquired through secondary endosymbiosis, supports carbon fixation, fatty acid synthesis and/or other metabolic needs in many other algal groups in ways that are consequential. For example, diatoms and haptophytes have strong biogeochemical effects; apicomplexans cause human disease (e.g., malaria); and dinoflagellates include both coral symbionts and toxin-producing "red tides" (1). The evolutionary processes that produced the Archaeplastida and secondary algal lineages remain under investigation (2-5), but it is clear that both nuclear and plastid genes from the ancestral red algae have contributed dramatically to broader eukaryotic evolution and diversity. Consequently, the imprint of red algal metabolism on the Earth's climate system, aquatic foodwebs, and human health is significant. Moreover, the oldest taxonomically resolved multicellular eukaryote in the fossil record (1.2 Ga) is the bangiophyte red alga *Bangiomorpha*, which closely resembles the extant marine alga *Bangia* (6). As is typical of bangiophytes, *Porphyra* grows in one of Earth's most physically stressful habitats, the intertidal zone, where organisms are exposed to daily and seasonally fluctuating temperatures, high levels of irradiance (including UV), and severe osmotic stress and desiccation. *Porphyra* and its ancestors have competed successfully in this dynamic and severe environment for over a billion years, through numerous changes in climate and mass extinctions.

Here we describe the genome of *Porphyra umbilicalis*. Examination of the *Porphyr*a genome and complete genomes of other red algae (*Chondrus crispus* (7); *Cyanidioschyzon merolae* (8)*; Galdieria sulphuria* (9); *Porphyridium purpureum* (10)*; Pyropia yezoensis* (11)) revealed numerous additional differences between the red algae and other eukaryotic lineages, including a reduced complement of motor proteins, unique signaling molecules, and augmented stress tolerance mechanisms, especially in *Porphyra*.

Results and Discussion

Genomic Analysis An 87.7 Mbp assembly of the *P. umbilicalis* (hereafter, *Porphyra*) nuclear genome was generated from PacBio whole genome shotgun (WGS) sequencing, with insertions and deletions corrected using Illumina WGS reads (SI Appendix, Methods, Tables S1-S2). The *Porphyra* genome has a substantial repeat component (43.9%) for a compact genome, with the

most common repeat classes being DNA (15.5 Mbp) and LTR (14.9Mbp) elements (SI Appendix, Table S5). Gene models were predicted at 13,125 loci using *de novo* gene prediction algorithms supported by evidence from protein homology and expression data (SI Appendix, Table S6). A typical gene has ~2 exons, implying abundant splicing for a red alga; however, only 235 alternative splice-forms were identified from expression data (SI Appendix, Table S6). Overall, the genome is 65.8% G+C, but protein coding regions average 72.9 %, and reach up to 94 % G+C (SI Appendix, Figs. S7, S8). Nearly 98% of the sequenced transcripts (ESTs) can be mapped to the genome assembly and a complete complement of genes encoding RNA polymerase subunits and other conserved informational proteins involved in transcription, translation, and DNA synthesis were identified (SI Appendix, Table S10), suggesting that the genome is nearly complete.

Phylogenomic analysis (12) of the red algae (Rhodophyta) demonstrates a class (Cyanidiophyceae) of extremophilic unicellular species and two sister clades of mesophilic species that we refer to here as the SCRP (Stylonematophyceae, Compsopogonophyceae, Rhodellophyceae, Porphyridiophyceae) and the BF (Bangiophyceae, Florideophyceae) (Fig. 1). The SCRP contains unicells, microscopic filaments, and microscopic blades; whereas, the BF clade holds macrophytes ("seaweeds") that comprise the majority of described species (13). Phylogenomic comparisons of Bangiophyceae (e.g., *Porphyra umbilicalis, Pyropia yezoensis*) and Florideophyceae (e.g., *Chondrus crispus*) suggest that these two red algal classes are highly diverged (SI Appendix, Fig. S11). The absence of some pathways and genes from red algae is likely due to genomic reduction in the red algal ancestor (4), and we confirmed that *Porphyra* lacks genes described previously as lost in other red algae including those encoding the glycosyl-phosphatidylinositol (GPI) anchor biosynthesis pathway (KEGG map00563, 22 genes), autophagy proteins (KO pathway ko04140, 17 genes), and most flagellar proteins (4).

Cytoskeleton The composition of the cytoskeleton must determine many of the capabilities and limitations of red algae, including *Porphyra,* because of the fundamental roles of the cytoskeleton in supporting intracellular movement of organelles and secretory vesicles, regulation of cell growth, size, and shape, and response to environmental signals including cell polarity (14). Cargoes (e.g., organelles, secretory vesicles) are typically transported along

microtubules (MTs) by plus-end directed kinesins and minus-end directed dyneins or along microfilaments (MFs) by myosins.

Actin-supported processes in red algae have been predicted from their inhibition by the actin inhibitor cytochalasin B (CB) and/or by correlated localizations of F-actin. The occurrence of cytoplasmic and mitotic spindle MTs are also reported (15, 16). However, despite the long-known absence of flagella and typical centrosomes (15), the red algal cytoskeleton remains poorly characterized (17), and our examination of cytoskeletal genes in *Porphyra* leads to conclusions that are both intriguing and puzzling. Genes encoding actin and tubulin are present in *Porphyra*, but the suites of regulatory proteins associated with MFs and MTs are notably reduced (SI Appendix, Tables S14, S15). Moreover, kinesin is the only motor protein that we were able to identify: both myosin and cytoplasmic dynein are absent from *Porphyra*. We find that the single myosin annotated previously (9) in the extremophile *Galdieria*, but absent from *Cyanidioschyzon* (8), is also found in three classes of the SCRP clade, but not in Porphyridiophyceae (SI Appendix, Fig. S16). The dynein motor seems to be absent in red algae except for a dynein heavy chain (HC) reported from *Chondrus crispus* that might be from a contaminant or horizontal gene transfer (SI Appendix Table S15). Kinesin is the only known motor protein that is found throughout the red algae (Fig. 2).

We identified four closely related actin genes in *Porphyra*, as well as the chromatin remodeling, actin-related protein Arp 4, but no other Arp proteins (SI Appendix, Table S14). The lack of the dynactin complex Arp 1 is consistent with loss of a dynein motor, but the general absence of Arp2/Arp3 from red algae is particularly surprising. In eukaryotes, Arp2/3 nucleates the formation of branched MFs that mediate amoeboid motion (14), which is observed in many types of red algal spores (18) including *P. umbilicalis* neutral spores (19) and *Pyropia pulchella* archeospores (20). How these spores move without Arp2/3 is an intriguing question; perhaps they rapidly polymerize MFs with the aid of other nucleating machinery to generate movement (14). Consistent with this idea, *Porphyra* and other red algae contain (SI Appendix, Tables S14-S15) members of the formin family, which nucleates MFs; profilin, which interacts with formins; cofilin, a key depolymerizing factor; and severin, which cuts MFs to promote remodeling. However, we did not find other well-conserved, widely distributed actin-modifying proteins

(e.g., WASP/WAVE, CapZ, fimbrin) in *Porphyra*, and few if any convincing homologs in other red algae (SI Appendix, Table S15).

The well-characterized, myosin-dependent cell streaming observed in some green algae (e.g., *Nitella*) is absent in red algae (17); however, myosin and MFs have roles in many eukaryotic organisms in slower movements of organelles and secretory vesicles and in cytokinesis (14). In unicellular Rhodellophyceae and Stylonematophyceae in the SCRP clade, MF-dependency was suggested based upon FITC-phallacidin-labeled MFs that ensheath secretory vesicles (21) while CB inhibited chloroplast rotation (22); these classes have the single myosin we identified within the SCR of the SCRP clade (SI Appendix, Fig. S16). In the Bangiophyceae and Florideophyceae, bundles of actin MFs were revealed with FITC-phalloidin or antibody labeling or described with transmission electron microscopy (16) in multiple species, including during spore migration (amoeboid movement), cytokinesis, and in the movement of sperm nuclei within the red algal egg cell (e.g. 17, 20, 23). These processes were suggested to be dependent upon actin MFs and myosin motors by demonstrations of appropriately located bundles of MFs coupled, in many cases, to inhibition by CB and a putative myosin inhibitor 2,3-butanedione monoxime (BDM). However, somehow, these MF-associated phenomena appear to occur in the absence of myosin, because we did not find myosin genes in bangiophyte and florideophyte genomes, and myosin's role was only inferred earlier (e.g. 20) through use of non-specific (24) myosin inhibitors (BDM).

Microtubules are found in the cytoplasm and particularly in the mitotic spindle of red algal nuclei (15, 16). We found that the *Porphyra* genome encodes the expected alpha and beta tubulin proteins, and some proteins related to tubulin folding (e.g., *Porphyra* contains cofactors B and D, but not A or E) and nucleation (e.g., gamma tubulin, gamma complex proteins; SI Appendix, Tables S14-15). However, many of the expected tubulin regulatory proteins widely distributed across eukaryotes, and even in other red algae, are missing. For example, *Porphyr*a contains EB1 and Mor1/XMAP215, two highly conserved proteins of the MT plus-end tracking (+TIP) complex, but the +TIP CLASP and the cross-linker MAP65 appear absent, even though these highly-conserved genes are found in other red algae (SI Appendix, S15). Unknown as yet

is whether *Porphyra* simply lacks these activities, or has recruited other proteins to fill their roles.

Like other red algae, *Porphyra* has lost dynein motors (HC) and intermediate chains (IC) but retains a particular light chain (LC) that is also conserved in plants, which independently lost flagellar motility (SI Appendix, Table S15). This dynein LC is expressed under abiotic stress and phytohormone treatments in plants (25); thus, it may have a role in the stress tolerance of *Porphyra*. In contrast to the loss of the dynein motor, *Porphyra* has representatives of kinesin subfamilies 5, 7, and 14, which are expected to be involved in spindle assembly, kinetochore function, and regulation of MT dynamics, respectively (Fig. 2, SI Appendix Tables S14-S15, Fig. S17). *Porphyra* also contains three divergent kinesins that usually group with the mitotic motor kinesin 13. Classical vesicle transport motors (kinesin subfamilies 1, 2, and 3) appear to be absent in *Porphyra*, which is particularly surprising considering the apparent loss of myosins and dynein. A few red algae do contain members of the kinesin 4 subfamily (Fig. 2), which are reported to be plus-end MT vesicle motors in plants (26), while kinesin 14, which is a minus-end directed transporter in plants (reviewed by 27, 28), is universally present in red algae (Fig. 2). Thus, kinesins 4 and 14 may provide a pair of motor proteins for moving vesicles and organelles along MTs in a few red algae, namely one of the extremophilic Cyanidiophyceae, *Galdieria*, and in the unicellular/microscopic SCRP clade (Fig. 2). However, unless kinesin 5 or kinesin 7 has unexpected functionality, *Porphyra* and the majority of other red algal species that are Bangiophyceae and Florideophyceae would also apparently lack a kinesin- MT motor system.

Based on our analysis of *Porphyra* and other red algae with sequenced nuclear genomes (*Chondrus* (7), *Cyanidioschyzon* (8)*, Galdieria* (29)*, Porphyridium* (10)*, Pyropia* (11)), the red algal cytoskeleton appears to lack the complexity and diversity of cytoskeletal elements present in other multicellular lineages (Figure 2). While it is possible that regulatory proteins are simply too divergent to recognize, the paucity of motors is especially apparent. We suggest that this observation could help to explain long-standing questions about morphological evolution in the red algae. Compared to members of other multicellular lineages (green algae/plants, brown algae, fungi, animals), both cell size and overall morphological size and complexity are limited in the red algae. For example, *P. umbilicalis* and other *Porphyra sensu lato* (30) typically have

small cylindrical cells (~ 10-40 μm long) that form thin haploid blades that are typically ≤ 25-50 cm long, only rarely reaching ≥ 1 m length (31, 32). The largest red algal cells are probably the ≤ 2 mm long cells of the filamentous florideophycean *Griffithsia* (33). In contrast, within the green algae/plants (Viridiplantae), some cells are millimeters-centimeters or even meters in length (1), and redwood trees grow to a height of 100 m. Moreover, some brown algae have millimeter-centimeter long cells, and kelps reach 46 m in length (31, 34); a few fungal cells are 15+ cm long (35), and hyphal individuals of basidiomycete fungi can reach > 50 m in length (36, 37); and, among the metazoans, blue whales have nerve cells that are 30 m long (38). Land plants independently lost cytoplasmic dynein, but they have an expanded set of kinesins that appear to be an evolutionary adaptation that partially compensates for their loss of the dynein motor (27). Significantly, plants, unlike most red algae, also have myosin motors (e.g., myosins 8, 11, Fig. 2), and some processes that are MT-dependent in other eukaryotic lineages are MF-dependent in higher plants. Gene knockouts of myosin 11 in *Arabidopsis* result in stunted growth, decreased expansion of leaf cells, and delays in flowering (39). In considering the small number of cytoskeletal elements in the red algae compared to those of other multicellular lineages (Fig. 2), we speculate that the limited cytoskeleton could have constrained the ability of red algae to develop large cells and larger, more complex individuals.

Stress The ecological success of *Porphyra* and closely related bangiophyceans (30) in the intertidal zone suggests that these 150+ species (40) have developed cellular mechanisms to cope with this harsh environment. In particular, *Porphyra* grows from the mid- high intertidal zone where it is routinely and repetitively exposed during daily low tides to high light, desiccation, and extreme fluctuations in temperature and salinity. Blades can lose up to 95% of their water on some days, but are metabolically active as soon as they are rehydrated by the rising tide (19). Here we infer novel adaptations to cope with these stresses.

Photoprotection Light is required for photosynthesis, but severe cellular damage can result from exposure of photosynthetic organisms to the high levels of light (visible and UV) that are present in the mid to high intertidal zone where most bangiacean algae including *Porphyra* grow. *Porphyra* has the same complement of genes (SI Appendix, Table S18) to carry out photosynthesis as other red algae, but exhibits a notable set of photoprotection strategies.

Among the 13 genes encoding chlorophyll a-binding light harvesting complex (LHC) proteins are two "RedCap" genes (41), which may be involved in reorganization of the photosynthetic antenna during the shift from darkness to light (42). *Porphyra* also has 11 genes encoding "high light induced" or "one-helix" proteins (here OHPs) that have essential roles for photoacclimation and cell viability (43, 44). Mechanistically, OHPs may regulate chlorophyll and tetrapyrrole biosynthesis, stabilize PSI, bind free free chlorophyll or chlorophyll breakdown products from damaged PSII complexes during the damage/repair cycle, and/or bind carotenoids that dissipate excess absorbed light energy. In contrast to the 10 *Porphyra* OHP genes, red algae from lower intertidal/subtidal positions and the plankton that have only 3-6 *OHP* genes (SI Appendix Table S18). The incomplete nature of some of these genomes means more analysis is needed, but the putative gene family expansion in *Porphyra* suggests positive selection for increased gene dosage. Although *Porphyra* was one of the first organisms in which quenching of excess excitation energy in response to desiccation stress was observed (45), the molecular mechanisms involved in quenching of excitation energy in red algae remain unclear.

Porphyra encodes genes for catalases and peroxidases, as well as the biosynthesis of numerous antioxidants such as ascorbic acid (vitamin C) and tocopherol (vitamin E) (SI Appendix, Tables S19-S21). When over-excitation of photosynthetic electron transport occurs and reactive oxygen is generated, catalase detoxifies hydrogen peroxide in red algae, and the expansion of the catalase gene family in *Porphyra* and *Pyropia* (5 genes, SI Appendix Table S20) compared to other red algae (1-2 genes) could reflect the demand for detoxification of reactive oxygen species (ROS) that cannot diffuse away from blades exposed by the falling tide to high light and air while they are still hydrated and photosynthetically active. Tocopherols prevent photooxidative damage of polyunsaturated fatty acids (46), and the γ-tocopherol methyl transferase (SI Appendix, Table S21) found encoded on the *Porphyra* genome catalyzes synthesis of α and β-tocopherol. The isomer composition is unknown, but α-tocopherol has been identified in *Porphyra* blades and is considered the more potent antioxidant (47, 48). The 32 heat shock proteins (HSP) in *Porphyra* indicate a possible expansion of the HSP40 family (SI Appendix, Table S19), which are co-chaperones of Hsp70 and play an important role in protein maturation and repair under normal and stressed conditions (49, 50).

Porphyra is frequently exposed to elevated intensities of UV radiation in the intertidal zone and shows remarkable tolerance to both UV-A and UV-B (51, 52). *Porphyra* has at least two strategies to protect photosynthesis and other key cellular processes from UV damage: novel mycosporine-like amino acids (MAA) and circadian control over the timing of sensitive processes in cells.

MAAs act as 'sunscreens' and comprise up to 1% of the dry weight of *Porphyra*, with the compound porphyra-334 being the major MAA (52). Four proteins, MysA, MysB, MysC, and MysD, support the biosynthesis of the MAA shinorine in cyanobacteria such as *Nostoc* (53, 54), whereas MysD is replaced by a non-ribosomal peptide synthase (NRPS) in *Anabaena* (Fig. 3). The enzyme catalyzing methylation of shinorine to produce porphyra-334 is still unknown. The *Porphyra* genome contains a gene encoding a MysA and MysB protein fusion that is also found in several other red algae that synthesize MAA, as well as in dinoflagellates, which were proposed to have acquired the still separate but neighboring genes from a cyanobacterium (55) (Figure 3; SI Appendix, Figs. S22-S23). The presence of the *MysA-MysB* fusion in red algae suggests that dinoflagellates could have acquired these genes from red algae through secondary or serial endosymbiosis (55) (SI Appendix, Figure S22). Moreover, *Porphyra* and related species contain a gene encoding a MysC-MysD fusion protein, and in the *P. umbilicalis* genome the *MysA-MysB* and *MysC-MysD* fusion genes are next to each other but transcribed on opposite DNA strands with adjacent 5'-ends (Fig. 3). Although the *Chondrus* genome also contains clustered *MysA-MysB* and *MysC-MysD*, the fusion proteins are encoded on opposite strands with the 3'-ends of the genes adjacent. Conservation of the MAA gene cluster in *Porphyra* and *Chondrus* and the two gene fusion events suggest that this arrangement provides a selective advantage and efficient MAA biosynthesis for red algae that experience high UV irradiance.

Developmental and abiotic stress responses are often associated with photoreceptors in eukaryotes. The plant circadian clock contains blue and red light photoreceptors including cryptochromes (CRY) and phytochromes (PHY) to entrain the circadian clock (56); these photoreceptors are also involved in other fundamental processes in plants, including growth and development. *Porphyra* does not appear to encode a PHY photoreceptor nor a typical plant *CRY* photoreceptor, although it has maintained three distinct *CRY* genes. The *Porphyra* CRY that is

most like plant CRYs is similar to a DNA photolyase (PHR2) (SI Appendix Table S25, Fig. S26) (57). *Porphyra* also has a CRY-DASH protein (58) (Pum2644s0001.1) in a separate subfamily of CRYs that display DNA repair activity and that are present from bacteria to vertebrates (58). In addition, there is an animal-like CRY (Pum0401s0002.1) encoded on the *Porphyra* genome (SI Appendix, Fig. S26). Animal-like CRYs could function as blue light photoreceptors associated with the entrainment of the circadian clock, as they do in *Drosophila* and some other insects, or could even add an oscillatory component to the clock as in mouse or humans (58). The *Porphyra* animal-like CRY is closely affiliated with the cryptochrome photolyase family (CPF, e.g., *Ostreococcus tauri*, *Phaeodactylum tricornutum* in SI Appendix, Fig. S26), which has maintained photolyase activity (59, 60) in contrast to other plant and animal-like CRYs. Moreover, CPF CRY can affect transcriptional activity in a heterologous clock system (e.g. mammalian CRY) and control blue-light dependent cellular processes, similar to the insect or plant CRYs (59, 60). Similar to the *Chlamydomonas reinhardtii* animal-like CRY (aCRY), the Pum0401s0002.1 protein could sense blue light and possibly other light qualities, including red light (61, 62); this red light sensing ability stems from the formation of the neutral radical form of the photoreceptor's flavin chromophore.

Some aspects of photosynthesis and central metabolism in *Porphyr*a may be under circadian or circatidal regulation. Furthermore, identification of genes involved in central metabolism (SI Appendix, Table S27) demonstrates that the *Porphyra* genome encodes enzymes necessary to support CAM (or C_4) metabolism, but there is currently no evidence for differential diurnal expression of these genes.

Signaling and Homeostasis *Porphyra* must cope with significant osmotic and ionic stress in the intertidal zone. Several low molecular weight carbohydrates act as compatible solutes in red algae including *Porphyra* (63-65). We found enzymes encoded on the *Porphyra* genome that support floridoside and isofloridoside synthesis, but no evidence for digeneaside synthesis (SI Appendix, Table S30). Taurine is a likely osmolyte in *Porphyra* (66), and we found homologs of enzymes such as cysteine dioxygenase implicated in metazoan taurine biosynthesis, which suggests an ancient origin for eukaryotic biosynthesis of this non-protein amino acid (SI Appendix, Table S30). *Porphyra* also has a wide range of ion transporters, including an unusual

class of Na^+/H^+ exchangers that are most similar to the NhaP class of transporters from α-Proteobacteria and are distinct from the Na^+/H^+ exchangers previously identified in eukaryotes. *Porphyra* has two P2C-type Na^+/K^+ ATPases and a P3A H^+-ATPase similar to those in land plants, suggesting *Porphyra* is able to energize its plasma membrane with either Na^+ or H^+ for secondary active transport, which could aid its survival in the intertidal zone (SI Appendix Table S30). The *Porphyra* genome also contains a range of Ca^{2+}-permeable membrane channels that could play a role in osmotic stress signaling, including several homologues of OSCA (SI Appendix S30), a recently identified Ca^{2+} channel in land plants (67); however, the Ca^{2+} sensor kinases through which *Porphyra* senses and responds to cytosolic Ca^{2+} elevations appear to be distinct from land plants and, indeed, from all other eukaryotes.

Land plants possess two expanded families of Ca^{2+} sensor kinases, the Ca^{2+}-dependent protein kinases (CDPKs) and the calcineurin B-like protein (CBL)-interacting protein kinases (CIPKs), which are activated through the binding of CBL (68, 69). The CIPKs have been extensively characterized in the green lineage (Viridiplantae), although they are present in diverse eukaryotes including stramenopiles, haptophytes and excavates, suggesting a likely origin early in eukaryotic evolution (70). Surprisingly, we found that genes encoding CIPKs and CBLs are absent from the *Porphyra* genome and from all other available red algal genomes and transcriptomes. The CDPKs are also absent from *Porphyra* and notably from other bangiophytes and florideophytes, but they are present in red algae in the SCRP clade (Fig. 4, SI Appendix Table S31). Another class of Ca^{2+} sensor kinases, the Ca^{2+}/calmodulin-dependent protein kinases (CAMKs), which are important in both plants and animals, are also missing in *Porphyra*. This suggests that much of the extensive network of Ca^{2+} sensor kinases found in other eukaryotes is absent in the red algae (Fig. 4). Whereas the *Porphyra* genome encodes several proteins with domains similar to the kinase domains of CAMKs, CDPKs and CIPKs, there is no indication that these proteins are regulated by Ca^{2+} or Ca^{2+}-binding proteins. Instead, we found that *Porphyra* possesses a novel class of Ca^{2+} sensor kinases with 2-3 Ca^{2+}-binding EF-hand domains at the N-terminus and a kinase domain at the C-terminus (Fig 4; SI Appendix, Table S32). The kinase domain from all known Ca^{2+} sensor kinases belongs to the CAMK group of kinases, whereas this uncharacterized protein belongs to the tyrosine kinase like (TKL) class of kinases (71, 72). The genes encoding homologous proteins are present on many other red algal genomes, including

Chondrus, *Cyanidioschyzon* and *Galdieria*, but appear to be absent from all other characterized eukaryotic genomes. Hence, the Ca^{2+}-dependent TKLs (CDTKLs) represent a newly recognized class of Ca^{2+}-regulated kinases that appear to be unique to red algae (Fig. 4).

The sucrose non-fermenting-1 (SNF1)-related kinase (SnRK) family of serine/threonine kinases plays important roles at the interface between metabolic and stress signaling from fungi (yeast) to land plants (73). There are 38 SnRKs divided into three sub-classes (SnRK1, 2, 3) in *Arabidopsis*, and the *Porphyra* genome encodes 31 proteins with homology to the kinase domains of *Arabidopsis* SnRK proteins (SI Appendix, Figure S33). Phylogenetic analysis places that *Porphyra* SnRK group in two clades that include *Arabidopsis* SnRK1 and SnRK3/CIPK, and a third expanded clade containing 19 unique members (SI Appendix, Fig S33). While no *Porphyra* SnRKs are most similar to the SnRK2 subfamily of *Arabidopsis,* several members of the SnRK3/CIPK clade contain motifs similar to the C-terminal domain II of SnRK2 (Fig S33B), which mediates interaction with PP2C in the abscisic acid (ABA) signaling pathway (74, 75). Furthermore, there are homologs of several ABA biosynthetic genes in *Porphyra* (SI Appendix, Fig S34A), and ABA synthesis and responses to exogenous ABA are reported for *Pyropia orbicularis* (76). The evidence suggests that the SnRK family plays important roles in stress responses including ABA-mediated responses in *Porphyra*. Genomic evidence also supports the presence of an ethylene pathway in *Porphyra*, because a number of ethylene biosynthetic genes were identified (SI Appendix Figure S34B), and ethylene has been detected and shown to induce stress responses in red algae (77).

The cell cycle is regulated by dimers of cyclins and cyclin dependent kinases (CDKs). Except in *C. merolae,* where circadian rhythms and stress responses regulate the G_1/S transition (78), the red algal cell cycle is not well characterized. Results here, coupled with the earlier studies of *C. merolae*, show that regulation of the red algal cell cycle is similar to that of metazoans and plants (79, 80) except that we did not find cyclin D encoded in any red algal genome, including that of *Porphyra* (SI Appendix, Table S35). Cyclin Ds are well conserved in all other eukaryotes where they regulate the G_1/S transition. The mitotic-specific cyclin A (CYCA), a known cell cycle progression regulator, might instead function in place of D-type

cyclins in red algae. We found that the glaucophyte (Archeoplastida) *Cyanophora paradoxica* (2) also lacks a cyclin D homolog.

Plant Defense Genes. Bangiophyte pathogens include some oomycetes, viruses, and bacteria, and these organisms sometimes cause serious economic losses to nori aquaculture. Land plants detect pathogens via an array of cell surface pattern recognition receptors (e.g. receptor like kinases) and intracellular receptors (e.g. nucleotide-binding domain and leucine-rich repeat proteins, NBS-LRR) (81). We found no evidence for this higher plant type of pathogen detection, but two families of intracellular ligand-binding protein containing NB-ARC domains, and a family of potential extracellular receptors containing malectin and immunoglobulin-like fold domains were found in the multicellular red algal genomes (SI Appendix Fig. S36). We also found a bangiophyte-specific family of at least five proteins harboring vWFA and C-type lectin domains (SI Appendix, Table S37); C-type lectin domains are involved in pathogen detection in some animals (82, 83).

Cell walls Red algae synthesize many unique polysaccharides such as agars that contribute to their ecological success and are of significant interest to biotechnological and industrial applications. The cells of the blade of *Porphyra* spp. form a non-rigid cell wall that plays an important role in osmotic acclimation by allowing dramatic changes in cell volume without plasmolysis (84, 85). The skeletal component of the cell wall of blade cells is not cellulose but partially crystalline beta-1,4-linked mannan in the outer cell wall, with a highly crystalline inner layer of beta-1,3-linked xylan (86, 87). We discovered two glycosyltransferase (GT2) enzymes (Fig. 5; SI Appendix Table S38) in *Porphyra* that are closely related to plant cellulose synthase-like CSLAs (mannan synthases) and CSLCs (xyloglucan synthases) and the green algal CSLs proposed to be implicated in mannan synthesis (88, 89). These *Porphyra* GT2 enzymes represent excellent candidates for mannan synthases, and their discovery in *Porphyra* suggests that both CSLA and mannan biosynthesis originated in the last common ancestor of green and red algae, rather than in the Viridiplantae as previously proposed (88). These findings suggest that mannans had an ancestral function in red algae, although it appears the Florideophyceae abandoned mannans for cellulose while the Bangiophyceae alternate between a mannan/xylan based cell wall in the gametophytes and a cellulose-based cell wall in the filamentous sporophytes. The

Porphyra genome has two genes encoding glycosyl hydrolases (GH) of the GH113 family, which are predicted to be beta-mannanases, indicating that *Porphyra* has the enzymes required for both the biosynthesis and degradation of mannans present in the cell walls of the blade.

The extracellular matrix (ECM) component of the cell wall in *Porphyra* is a highly hydrated agar called porphyran that limits desiccation and contributes to the flexibility of the cell wall during osmotic stress (90) (SI Appendix, Table S38, Figs. S39, S43). Porphyran is mainly composed of porphyranobiose but also contains varying percentages of agarobiose (91). The *Porphyra* genome encodes a family 2 carbohydrate sulfotransferase (ST2) that may act as a L-galactose-6-O-sulfotransferase in the biosynthesis of porphyran (SI Appendix S38). After *C. crispus*, the closest related homologs to this red algal sulfotransferase are found in metazoans (*Nematostella vectensis*, sea anemones) and the closest characterized enzymes are dermatan and chondroitin 4-O-sulfotransferases; likewise, the *Porphyra* GT7, after the *C. crispus* GT7, is most similar to metazoan GT7s (*Acropora digitifera*, staghorn corals) and the closest characterized enzyme is chondroitin sulfate N-acetylgalactosaminyltransferase 2 (*H. sapiens*). These findings lend support to the ancestral nature of the biosynthesis of sulfated polysaccharides in the eukaryotes. Homologous carbohydrate sulfotransferases are indeed conserved at least in metazoans, brown algae, and red algae, so were most likely present in the last common eukaryotic ancestor (LECA) (90, 92). Genes encoding carbohydrate sulfotransferases are not found in sequenced genomes of terrestrial plants and freshwater algae, but marine angiosperms have, relatively recently, readapted to the marine environment by developing an ECM containing sulfated polysaccharides through an unknown mechanism of convergent evolution (93). This speaks to the critical role of sulfated ECMs in high salinity environments (physiological saline and seawater). The *Porphyra* genome encodes three GH16 enzymes that form a clade with other red algae and with the agarases and porphyranases from marine bacteria (SI Appendix, Figure S39). These enzymes likely function in cell wall remodeling and are unknown in unicellular red algae, which supports the possible involvement of bacteria in the evolution of multicellular algae (94).

Obtaining nutrients at high tide. We found that *Porphyra* has genes for Fe-uptake mechanisms that likely lend specificity and enable high-affinity assimilation during the narrow tidal window

when blades are underwater (Fig. 6; SI Appendix, Table S44). The identified proteins encoded on the *Porphyra* genome include a high-affinity iron transport complex containing a permease (FTR1) and multicopper oxidase (FET3 in yeast/FOX1 in green algae) and members of the FEA and ISIP2a families. The later are related algal protein families containing secreted soluble proteins involved in iron assimilation and membrane-bound iron uptake facilitators (95, 96). Given the presence of FTR1 in the three main Archaeplastida lineages (Viridiplantae, Glaucophyta and Rhodophyta), we hypothesize that this permease was encoded in the last common Archaeplastida ancestor but was lost after the transition to land when plants evolved true roots (Fig. 6). We also found that the presence of the FEA/ISIP2a-like proteins correlated with the presence of cytochrome c_6 (CYC6), an iron-dependent electron transfer protein in the chloroplast, which like FTR1 was also lost after the transition to land (Fig. 6). Other inorganic and organic nutrient uptake and conversion capabilities appear similar to those described for other red algae (e.g., Fig. 6; SI Appendix, Tables S48, S50, S51, S49) except that the number of ammonium transporters (AMT; 7 genes, SI Appendix Table S47) and intracellular copper transporters (P_{1B}-type ATPases; 6 genes (*MTA1-6*), SI Appendix Table S44 and Figure S45) is greater in *Porphyra*, perhaps reflecting the metabolic demands of life in the upper intertidal zone.

Porphyra ("laver") and related genera (i.e., *Pyropia,* "nori") are commercially important human foods based upon their high mineral, protein, and vitamin content (97) (See Fig. 6; SI Appendix Figs. S49, S57, Tables S53, S58 for related findings). No eukaryote synthesizes vitamin B_{12} (cobalamin), but *Porphyra* is a rich source of this organic micronutrient. Vitamin B_{12} is a required cofactor for B_{12}-dependent methionine synthase (METH), and *Porphyra* encodes both METH and METE, the latter a B_{12}-independent isoform of methionine synthase (98). Methionine synthase plays a vital role in linking the folate cycle, essential for DNA metabolism, and the methylation cycle responsible for production of S-adenosylmethionine, the universal methyl donor. The use of METH implies that *Porphyra* takes up B_{12} from its abundant bacterial epiphytes (94), and we find here that *Porphyra*, unlike other sequenced red algae, encodes the full pathway required to remodel pseudocobalamin, produced by cyanobacteria (99), to cobalamin, the particular chemical variant of B_{12} that is bioactive for eukaryotic algae, and indeed humans (SI Appendix, Table S58). In *Chlamydomonas*, *METE* expression was found to be suppressed under environmentally relevant levels of heat stress, and survival was dependent

upon thermally stable METH (100); the fact that *Porphyra* is frequently heat-stressed in its upper intertidal habitat might similarly explain why it retains both isoforms of this enzyme. Interestingly, the nutritional value of *Porphyra* appears to be directly related to the nutrient requirements for survival in a harsh habitat.

Summary

The red algae have long commanded attention because of the 1.2 Ga age of the multicellular bangiophytes, the unique intricacies of their life histories, and their economic importance, but genomic analysis of *Porphyra* sharpens our understanding of just how different the red algae are from other eukaryotes. Because the cytoskeleton is so central to growth, development, and the ability to respond to environmental signals, the paucity of cytoskeletal elements in *Porphyra* and other red algae is striking. Only kinesin motor proteins are universally present; dynein motors are absent, and most of the red algae, including *Porphyra*, appear to lack myosin. This minimal cytoskeleton offers a potential explanation for the extreme reduction in cell size and adult stature of red algae compared to other multicellular lineages. The unique calcium-dependent signaling pathway further suggests that *Porphyra* may use distinctive mechanisms to sense and respond to its environment. Most bangiophytes including *Porphyra* live under harsh intertidal conditions. Our discovery of ancestral mechanisms of cell wall formation, an expanded array of UV/high light/ROS/thermal protection strategies, and a wealth of nutrient transporters encoded by the *Porphyra* genome helps to explain how these red algae have thrived for over a billion years in the pounding waves, baking sun, and drying winds of the high intertidal zone.

Methods

P. umbilicalis was isolated into unialgal culture from a haploid blade growing at Schoodic Point, Maine (44°20'1.68" N; 68°3'29.14" W) on April 3, 2008. This single genotype (101) was cloned by growth of material from asexual neutral spores, followed by DNA extraction, and assembly of the genome from sequences produced using Illumina and Pac-Bio sequencing platforms (see SI Appendix, Methods for full details).

Acknowledgements We are grateful to the many colleagues who provided useful discussion as participants in the *Porphyra* Research Coordination Network (NSF RCN 0741907 to SHB, EG,

ARG, JS), especially to Elisabeth Gantt (Univ. Maryland). Sequencing and assembly of the *Porphyra* genome were provided by the Joint Genome Institute (U. S. Dept. of Energy) under a Community Science Program award from the Office of Science of the U. S. Dept of Energy under Contract No. DE-AC02-05CH11231 (to SHB, EG, ARG, JS). Other major research support was provided by NSF 0929558 (to SHB, ARG), and NOAA contract NA060AR4170108 (to SHB). Additional acknowledgements appear in the SI Appendix.

References

1. Graham LE *et al.* (2016) *Algae* (LJLM Press, Madison, WI) 3rd ed.
2. Price DC *et al.* (2012) *Cyanophora paradoxa* genome elucidates origin of photosynthesis in algae and plants. *Science* 335:843-847.
3. Stiller JW *et al.* (2014) The evolution of photosynthesis in chromist algae through serial endosymbioses. *Nat Commun* 5:5764.
4. Qiu H *et al.* (2015) Evidence of ancient genome reduction in red algae (Rhodophyta). *J Phycol* 51:624–636.
5. Burki F *et al.* (2016) Untangling the early diversification of eukaryotes: a phylogenomic study of the evolutionary origins of *Centrohelida*, *Haptophyta* and *Cryptista*. *Proc R Soc Biol Sci* 283.
6. Butterfield N (2000) *Bangiomorpha pubescens* n gen n sp: Implications fore the evolution of sex, multicellularity, and the Mesoproterozoic/Neoproterozoic radiation of eukaryotes. *Paleobiology* 26:383-404.
7. Collén J *et al.* (2013) Genome structure and metabolic features in the red seaweed *Chondrus crispus* shed light on evolution of the Archaeplastida. *Proc Natl Acad Sci USA* 110:5247-5252.
8. Matsuzaki M *et al.* (2004) Genome sequence of the ultrasmall unicellular red alga *Cyanidioschyzon merolae* 10D. *Nature* 428:653-657.
9. Schonknecht G *et al.* (2013) Gene transfer from bacteria and archaea facilitated evolution of an extremophilic eukaryote. *Science* 339:1207-1210.
10. Bhattacharya D *et al.* (2013) Genome of the red alga *Porphyridium purpureum*. *Nat Commun* 4:1941.

11. Nakamura Y *et al.* (2013) The first symbiont-free genome sequence of marine red alga, Susabi-nori (*Pyropia yezoensis*). *PLoS One* 8:e57122.
12. Qiu H, Yoon HS & Bhattacharya D (2016) Red algal phylogenomics provides a robust framework for inferring evolution of key metabolic pathways. *PLoS Currents* 8:e4567.
13. Guiry MD (2012) How many species of algae are there? *J Phycol* 48:1057-1063.
14. Alberts B *et al.* (2014) *Molecular Biology of the Cell* (Garland Science, New York) 6th ed.
15. Pueschel C (1990) Cell Structure. *Biology of the Red Algae*, eds Cole K & Sheath R (Cambridge University Press, Cambridge), pp 7-42.
16. Babuka SJ & Pueschel CM (1998) A freeze-substitution ultrastructural study of the cytoskeleton of the red alga *Antithamnion kylinii* (Ceramiales). *Phycologia* 37:251-258.
17. Yoon HS *et al.* (2016) Rhodophyta. *Handbook of the Protists*, eds Archibald JM, Simpson AG, & Slamovits CH (Springer International Publishing, Switzerland), pp 1-43.
18. Pickett-Heaps J *et al.* (2001) Time-lapse videomicroscopy of cell (spore) movement in red algae. *Eur J Phycol* 36:9-22.
19. Blouin NA *et al.* (2011) *Porphyra*: a marine crop shaped by stress. *Trends Plant Sci* 16:29-37.
20. Ackland JC, West JA & Pickett-Heaps J (2007) Actin and myosin regulate pseudopodia of *Porphyra pulchella* (Rhodophyta) archeospores. *J Phycol* 43:129-138.
21. Wilson SM, Pickett-Heaps JD & West JA (2006) Vesicle transport and the cytoskeleton in the unicellular red alga *Glaucosphaera vacuolata*. *Phycol Res* 54:15-20.
22. Wilson S *et al.* (2002) Chloroplast rotation and morphological plasticity of the unicellular alga *Rhodosorus* (Rhodophyta, Stylonematales). *Phycol Res* 50:183-191.
23. Wilson SM, Pickett-Heaps JD & West JA (2002) Fertilization and the cytoskeleton in the red alga *Bostrychia moritziana* (Rhodomelaceae, Rhodophyta). *Eur J Phycol* 37:509-522.
24. Ostap EM (2002) 2,3-Butanedione monoxime (BDM) as a myosin inhibitor. *J Muscle Res & Cell Motil* 23:305-308.
25. Cao J, Li X & Lu Y (2017) Dynein light chain family genes in 15 plant species: Identification, evolution and expression profiles. *Plant Sci* 254:70-81.

26. Kong Z *et al.* (2015) Kinesin-4 functions in vesicular transport on cortical microtubules and regulates cell wall mechanics during cell elongation in plants. *Mol Plant* 8:1011-1023.
27. Li J, Xu Y & Chong K (2012) The novel functions of kinesin motor proteins in plants. *Protoplasma* 249:95-100.
28. Jonsson E *et al.* (2015) Clustering of a kinesin-14 motor enables processive retrograde microtubule-based transport in plants. *Nat Plants* 1:15087.
29. Barbier G *et al.* (2005) Comparative genomics of two closely related unicellular thermo-acidophilic red algae, *Galdieria sulphuraria* and *Cyanidioschyzon merolae*, reveals the molecular basis of the metabolic flexibility of *Galdieria sulphuraria* and significant differences in carbohydrate metabolism of both algae. *Plant Physiol* 137:460-474.
30. Sutherland JE *et al.* (2011) A new look at an ancient order: Generic revision of the Bangiales (Rhodophyta). *J Phycol* 47:1131-1151.
31. Abbott IA, Isabella A & Hollenberg GJ (1992) *Marine Algae of California* (Stanford University Press) pp 827.
32. Sears J (2002) *NEAS Keys to Benthic Marine Algae of the Northwestern Coast of North America* (Northeast Algal Society, Fall River, MA) 2nd ed pp 161.
33. Goff LJ & Coleman AW (1987) The solution to the cytological paradox of isomorphy. *J Cell Biol* 104:739-748.
34. Fritsch F (1945) *The Structure and Reproduction of the Algae Vol II* (Cambridge University Press, Cambridge) pp 939.
35. Bergman K *et al.* (1969) Phycomyces. *Bacteriol Rev* 33:99-157.
36. Smith ML, Bruhn JN & Anderson JB (1992) The fungus *Armillaria bulbosa* is among the largest and oldest living organisms. *Nature* 356:428-431.
37. Travadon R *et al.* (2012) Inferring dispersal patterns of the generalist root fungus *Armillaria mellea*. *New Phytologist* 193:959-969.
38. Smith DH (2009) Stretch growth of integrated axon tracts: Extremes and exploitations. *Prog Neurobiol* 89:231-239.
39. Peremyslov VV, Prokhnevsky AI & Dolja VV (2010) Class XI myosins are required for development, cell expansion, and f-actin organization in *Arabidopsis*. *Plant Cell* 22:1883-1897.

40. Guillemin ML *et al.* (2016) The bladed Bangiales (Rhodophyta) of the South Eastern Pacific: Molecular species delimitation reveals extensive diversity. *Mol Phylogenet Evol* 94:814-826.
41. Engelken J, Brinkmann H & Adamska I (2010) Taxonomic distribution and origins of the extended LHC (light-harvesting complex) antenna protein superfamily. *BMC Evol Biol* 10:233.
42. Sturm S *et al.* (2013) A novel type of light-harvesting antenna protein of red algal origin in algae with secondary plastids. *BMC Evol Biol* 13:159.
43. He Q *et al.* (2001) The high light-inducible polypeptides in *Synechocystis* PCC6803. Expression and function in high light. *J Biol Chem* 276:306-314.
44. Komenda J & Sobotka R (2016) Cyanobacterial high-light-inducible proteins--Protectors of chlorophyll-protein synthesis and assembly. *Biochim Biophys Acta* 1857:288-295.
45. Bose S, Herbert SK & Fork DC (1988) Fluorescence characteristics of photoinhibition and recovery in a sun and a shade species of the red algal genus *Porphyra*. *Plant Physiol* 86:946-950.
46. Collakova E & DellaPenna D (2003) The role of homogentisate phytyltransferase and other tocopherol pathway enzymes in the regulation of tocopherol synthesis during abiotic stress. *Plant Physiol* 133:930-940.
47. Kamal-Eldin A & Appelqvist LA (1996) The chemistry and antioxidant properties of tocopherols and tocotrienols. *Lipids* 31:671-701.
48. Ferraces-Casais P *et al.* (2011) Evaluation of bioactive compounds in fresh edible seaweeds. *Food Anal Methods* 5:828–834.
49. Walsh P *et al.* (2004) The J-protein family: modulating protein assembly, disassembly and translocation. *EMBO Rep* 5:567-571.
50. Liberek K, Lewandowska A & Zietkiewicz S (2008) Chaperones in control of protein disaggregation. *EMBO J* 27:328-335.
51. Dring MJ *et al.* (1996) Sesnsitivity of intertidal and subtidal red algae to UVA and UVB radiation, as monitored by chlorophyll measurements: Influence of collection depth and season and length of irradiation. *Eur J Phycol* 31:293-302.

52. Gröninger A, Hallier C & Häder D-P (1999) Influence of UV radiation and visible light on *Porphyra umbilicalis*: photoinhibition and MAA concentration. *J Appl Phycol* 11:437–445.
53. Balskus EP & Walsh CT (2010) The genetic and molecular basis for sunscreen biosynthesis in cyanobacteria. *Science* 329:1653-1656.
54. Gao Q & Garcia-Pichel F (2011) Microbial ultraviolet sunscreens. *Nat Rev Microbiol* 9:791-802.
55. Waller RF, Slamovits CH & Keeling PJ (2006) Lateral gene transfer of a multigene region from cyanobacteria to dinoflagellates resulting in a novel plastid-targeted fusion protein. *Mol Biol Evol* 23:1437-1443.
56. Harmer SL (2009) The circadian system in higher plants. *Ann Rev Plant Biol* 60:357-377.
57. Petersen JL, Lang DW & Small GD (1999) Cloning and characterization of a class II DNA photolyase from *Chlamydomonas*. *Plant Mol Biol* 40:1063-1071.
58. Chaves I *et al.* (2011) The cryptochromes: blue light photoreceptors in plants and animals. *Annu Rev Plant Biol* 62:335-364.
59. Coesel S *et al.* (2009) Diatom PtCPF1 is a new cryptochrome/photolyase family member with DNA repair and transcription regulation activity. *EMBO reports* 10:655-661.
60. Heijde M *et al.* (2010) Characterization of two members of the cryptochrome/photolyase family from Ostreococcus tauri provides insights into the origin and evolution of cryptochromes. *Plant, Cell & Environ* 33:1614-1626.
61. Beel B *et al.* (2013) News about cryptochrome photoreceptors in algae. *Plant Signal Behav* 8:e22870.
62. Oldemeyer S *et al.* (2016) Essential role of an unusually long-lived tyrosyl radical in the response to red light of the animal-like Cryptochrome aCRY. *J Biol Chem* 291:14062-14071.
63. Karsten U, Barrow KD & King RJ (1993) Floridoside, L-Isofloridoside, and D-Isofloridoside in the red alga *Porphyra columbina* (Seasonal and osmotic effects). *Plant Physiol* 103:485-491.
64. Pade N *et al.* (2015) Floridoside and isofloridoside are synthesized by trehalose 6-phosphate synthase-like enzymes in the red alga *Galdieria sulphuraria*. *New Phytol* 205:1227-1238.

65. Qian F *et al.* (2015) The littoral red alga *Pyropia haitanensis* uses rapid accumulation of floridoside as the desiccation acclimation strategy. *J Appl Phycol* 27:621-632.
66. Ye Y *et al.* (2013) Metabolic phenotypes associated with high-temperature tolerance of *Porphyra haitanensis* strains. *J Agric Food Chem* 61:8356-8363.
67. Yuan F *et al.* (2014) OSCA1 mediates osmotic-stress-evoked Ca2+ increases vital for osmosensing in *Arabidopsis*. *Nature* 514:367-371.
68. Weinl S & Kudla J (2009) The CBL-CIPK Ca^{2+}-decoding signaling network: function and perspectives. *New Phytol* 184:517-528.
69. Edel KH & Kudla J (2015) Increasing complexity and versatility: how the calcium signaling toolkit was shaped during plant land colonization. *Cell Calcium* 57:231-246.
70. Beckmann L *et al.* (2016) A calcium sensor – protein kinase signaling module diversified in plants and is retained in all lineages of Bikonta species. *Scientific Reports* 6:31645.
71. Hui R, El Bakkouri M & Sibley LD (2015) Designing selective inhibitors for calcium-dependent protein kinases in apicomplexans. *Trends Pharmacol Sci* 36:452-460.
72. Martin DM, Miranda-Saavedra D & Barton GJ (2009) Kinomer v. 1.0: a database of systematically classified eukaryotic protein kinases. *Nucleic Acids Res* 37:D244-250.
73. Coello P, Hey SJ & Halford NG (2011) The sucrose non-fermenting-1-related (SnRK) family of protein kinases: potential for manipulation to improve stress tolerance and increase yield. *J Exp Bot* 62:883-893.
74. Cutler SR *et al.* (2010) Abscisic acid: emergence of a core signaling network. *Annu Rev Plant Biol* 61:651-679.
75. Xie T *et al.* (2012) Molecular mechanism for inhibition of a critical component in the Arabidopsis thaliana abscisic acid signal transduction pathways, SnRK2.6, by protein phosphatase ABI1. *J Biol Chem* 287:794-802.
76. Guajardo E, Correa JA & Contreras-Porcia L (2016) Role of abscisic acid (ABA) in activating antioxidant tolerance responses to desiccation stress in intertidal seaweed species. *Planta* 243:767-781.
77. Uji T *et al.* (2016) Ethylene regulation of sexual reproduction in the marine red alga *Pyropia yezoensis* (Rhodophyta). *J Appl Phycol* 28:3501-3509.

78. Kobayashi Y *et al.* (2016) Abscisic acid participates in the control of cell cycle initiation through heme homeostasis in the unicellular red alga *Cyanidioschyzon merolae*. *Plant Cell Physiol* 57:953-960.
79. Bisova K, Krylov DM & Umen JG (2005) Genome-wide annotation and expression profiling of cell cycle regulatory genes in *Chlamydomonas reinhardtii*. *Plant Physiol* 137:475-491.
80. Moriyama T *et al.* (2010) Characterization of cell-cycle-driven and light-driven gene expression in a synchronous culture system in the unicellular rhodophyte *Cyanidioschyzon merolae*. *Microbiology* 156:1730-1737.
81. Dangl JL & Jones JD (2001) Plant pathogens and integrated defence responses to infection. *Nature* 411:826-833.
82. O'Rourke D *et al.* (2006) Genomic clusters, putative pathogen recognition molecules, and antimicrobial genes are induced by infection of *C. elegans* with *M. nematophilum*. *Genome Res* 16:1005-1016.
83. Hollmig ST, Ariizumi K & Cruz PD, Jr. (2009) Recognition of non-self-polysaccharides by C-type lectin receptors dectin-1 and dectin-2. *Glycobiology* 19:568-575.
84. Reed RH, Collins JC & Russell G (1980) The effects of salinity upon cellular volume of the marine red alga *Porphyra purpurea* (Roth) C. Ag. *J Exp Bot* 31:1521-1537.
85. Wiencke C & Lauchli A (1980) Growth, cell volume, and fine structure of *Porphyra umbilicalis* in relation to osmotic tolerance. *Planta* 150:303-311.
86. Baldan B *et al.* (1995) Polysaccharide localization in the cell-wall of *Porphyra leucosticta* (Bangiophyceae, Rhodophyta) during the life-cycle. *Bot Mar* 38:31-36.
87. Mukai LS, Craigie JS & Brown RG (1981) Chemical composition and structure of the cell walls of the conchocelis and thallus phases of *Porphyra tenera* (Rhodophyceae). *J Phycol* 17:192-198.
88. Yin Y, Huang J & Xu Y (2009) The cellulose synthase superfamily in fully sequenced plants and algae. *BMC Plant Biol* 9:99.
89. Popper ZA & Tuohy MG (2010) Beyond the green: understanding the evolutionary puzzle of plant and algal cell walls. *Plant Physiol* 153:373-383.

90. Ficko-Blean E, Hervé C & Michel G (2015) Sweet and sour sugars from the sea: the biosynthesis and remodeling of sulfated cell wall polysaccharides from marine macroalgae. *Perspect Phycol* 2:51-64.
91. Rees DA & Conway E (1962) The structure and biosynthesis of porphyran: a comparison of some samples. *Biochem J* 84:411-416.
92. Michel G *et al.* (2010) The cell wall polysaccharide metabolism of the brown alga *Ectocarpus siliculosus*. Insights into the evolution of extracellular matrix polysaccharides in eukaryotes. *New Phytol* 188:82-97.
93. Olsen JL *et al.* (2016) The genome of the seagrass *Zostera marina* reveals angiosperm adaptation to the sea. *Nature* 530:331-335.
94. Miranda LN *et al.* (2013) Diversity and abundance of the bacterial community of the red macroalga *Porphyra umbilicalis*: did bacterial farmers produce macroalgae? *PLoS One* 8:e58269.
95. Allen MD *et al.* (2007) FEA1, FEA2, and FRE1, encoding two homologous secreted proteins and a candidate ferrireductase, are expressed coordinately with FOX1 and FTR1 in iron-deficient *Chlamydomonas reinhardtii*. *Eukaryot Cell* 6:1841-1852.
96. Morrissey J *et al.* (2015) A novel protein, ubiquitous in marine phytoplankton, concentrates iron at the cell surface and facilitates uptake. *Curr Biol* 25:364-371.
97. Wells ML *et al.* (2016) Algae as nutritional and functional food sources: revisiting our understanding. *J Appl Phycol*:1-34.
98. Helliwell KE *et al.* (2011) Insights into the evolution of vitamin B_{12} auxotrophy from sequenced algal genomes. *Mol Biol Evol* 28:2921-2933.
99. Helliwell KE *et al.* (2016) Cyanobacteria and eukaryotic algae use different chemical variants of vitamin B_{12}. *Curr Biol* 26:999-1008.
100. Xie B *et al.* (2013) *Chlamydomonas reinhardtii* thermal tolerance enhancement mediated by a mutualistic interaction with vitamin B(12)-producing bacteria. *ISME J* 7:1544-1555.
101. Blouin NA & Brawley SH (2012) An AFLP analysis of clonality in widespread asexual populations of *Porphyra umbilicalis* (Rhodophyta) with a sensitivity analysis for bacterial contamination. *Marine Biology* 159:2723-2729.
102. Kollmar M (2016) Fine-tuning motile cilia and flagella: evolution of the dynein motor proteins from plants to humans at high resolution. *Mol Biol Evol* 33:3249-3267.

103. Wickstead B, Gull K & Richards TA (2010) Patterns of kinesin evolution reveal a complex ancestral eukaryote with a multifunctional cytoskeleton. *BMC Evol Biol* 10:110.
104. Odronitz F & Kollmar M (2007) Drawing the tree of eukaryotic life based on the analysis of 2,269 manually annotated myosins from 328 species. *Genome Biol* 8:R196.

Figure Legends

Figure 1. Phylogenomic-based classification of the Rhodophyta reveals a basal clade of extremophilic species (Cyanidiophyceae) and two sister clades (herein: SCRP, BF) of mesophilic species (12, 17). Most described red algal species belong to the BF clade; note position of *Porphyra*. Red shading of SCRP and BF is arbitrary, but blue-green is used for Cyanidiophyceae because members lack phycoerythrin as an accessory photosynthetic pigment and are blue-green.

Figure 2. Distribution of cytoskeletal motor proteins (kinesin, cytoplasmic dynein, and myosin) in red algae compared to selected other groups of Eukarya, showing the reduced cytoskeletal capacity of red algal cells. Standard motor family nomenclature for dyneins (102) kinesins (103) and myosins (104) are used; for example, kinesins K1, K2, and K3 are cytoskeletal motors that move organelles and vesicles in many eukaryotes. Footnotes: *This may be a contaminating sequence †The *Saccharomyces* dynein complex has lost much of the functionality found in other organisms (102) ‡ Additional, flagellar-related dynein HC are not shown and are absent in red algae and many plants. §Myosins M8 and/or M11 are vesicle transporters in plants ¶G*aldieria* and SCR members of the SCRP clade (Fig 1) have a myosin similar to myosin 27 of apicomplexans

Figure 3. Conserved gene clusters involved in mycosporine-like amino acid (MAA) biosynthesis in *Porphyra umbilicalis* and related red algae**.** (A) Biosynthetic pathway from sedoheptulose 7-phosphate to shinorine in cyanobacteria and to porphyra-334 in red algae. (B) Comparison of gene clusters and gene fusions in the cyanobacteria *Anabaen*a and *Nostoc* and the red algae *P. umbilicalis* and *Chondrus crispus.*

Figure 4. Module structure and presence or absence of different Ca^{2+} sensor kinases in *Porphyr*a and other eukaryotes (Ca^{2+}-calmodulin-dependent kinases [CAMK], calcineurin B-like protein kinases [CIPK], Ca^{2+}-dependent protein kinases [CDPK], and Ca^{2+}-dependent tyrosine-like kinases [CDTKL]). The CDTKLs from *Porphyra* and other red algae represent a newly recognized family of protein kinases belonging to the TKL (tyrosine kinase-like) family that contains multiple Ca^{2+} -binding EF hands.

Figure 5. Neighbor-joining phylogenetic tree of glycosyltransferase family 2 (GT2) showing the positions of *Porphyra* sequences (dark triangles) and rooted on bacterial bcsA. Red algal cellulose synthases (CESA) cluster closest to stramenopile oomycete CESAs, while bangiophyte cellulose synthase-like (CSL) enzymes are sister to a clade of green plant CSLs that includes a mannan synthase. Two red algal GT2s are related to bacterial cellulose synthase (bcsA)), likely due to horizontal gene transfer. Red algal sequences lie on red branches and triangles group sequences in Viridiplantae (green); stramenopiles (brown); Cyanobacteria (turquoise;) Bacteria (pink); and Amoebozoa (gray). The proteins were aligned using MAFFT with the L-INS-I algorithm and the scoring matrix Blosum62 and then manually refined with BIOEDIT. The phylogenetic tree was calculated using maximum likelihood in MEGA 6.06. See SI Appendix Table S41 for full names and GenBank accession numbers. Bootstrap values ≥ 65% are shown at nodes.

Figure 6. Identification of homologs and co-occurrence of selected metal transporters, metaldependent proteins, photosynthesis, multicellularity and/or absence of true roots. A filled circle signifies the presence of at least one homolog of the indicated protein family (FTR1, high-affinity iron permease; CYC6, cytochrome c6; FEA/ISIP2a, Fe-assimilation proteins; PCY, plastocyanin). The coloring denotes co-occurrence of that protein family with a trait of the same color, while a blue inner circle (in the case of PCY) indicates that the protein family is missing from available red algal genomes. A schematic species tree is shown at the left.

Figure 1

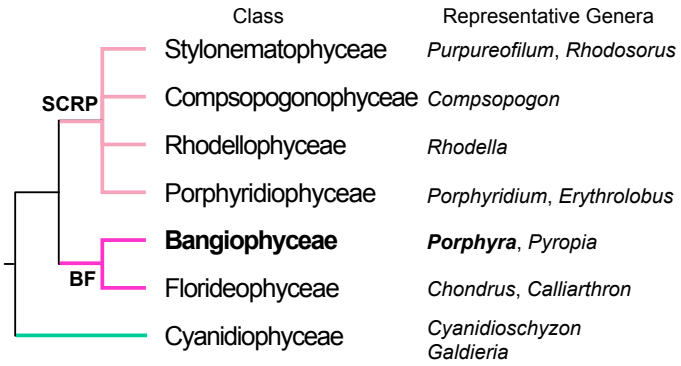

Class
Representative Genera
Stylonematophyceae
Purpureofilum, Rhodosorus
SCRP
Compsopogonophyceae
Compsopogon
Rhodellophyceae
Rhodella
Porphyridiophyceae
Porphyridium, Erythrolobus
Bangiophyceae
Porphyra, Pyropia
BF
Florideophyceae
Chondrus, Calliarthron
Cyanidiophyceae
Cyanidioschyzon
Galdieria

Figure 2

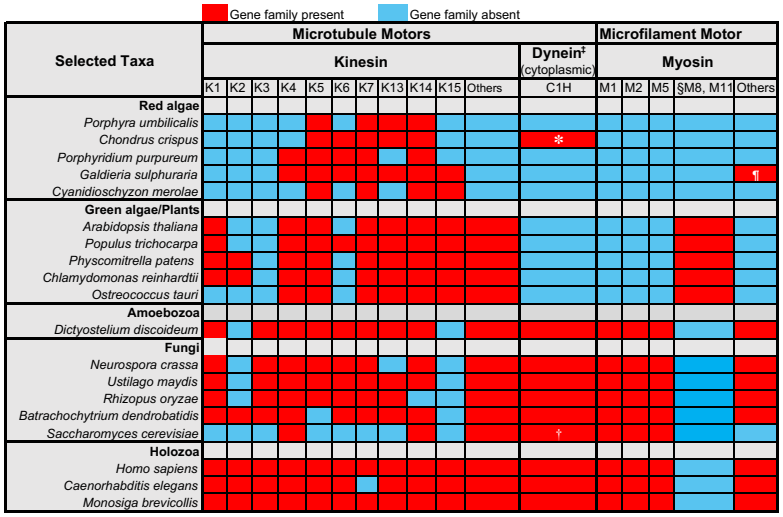

Gene family present
Gene family absent
Selected Taxa
Microtubule Motors
Kinesin
Dynein‡ (cytoplasmic)
Microfilament Motor
Myosin
K1 K2 K3 K4 K5 K6 K7 K13 K14 K15 Others
C1H
M1 M2 M5 §M8, M11 Others
Red algae
Porphyra umbilicalis
Chondrus crispus
Porphyridium purpureum
Galdieria sulphuraria
Cyanidioschyzon merolae
Green algae/Plants
Arabidopsis thaliana
Populus trichocarpa
Physcomitrella patens
Chlamydomonas reinhardtii
Ostreococcus tauri
Amoebozoa
Dictyostelium discoideum
Fungi
Neurospora crassa
Ustilago maydis
Rhizopus oryzae
Batrachochytrium dendrobatidis
Saccharomyces cerevisiae
Holozoa
Homo sapiens
Caenorhabditis elegans
Monosiga brevicollis

Figure 3

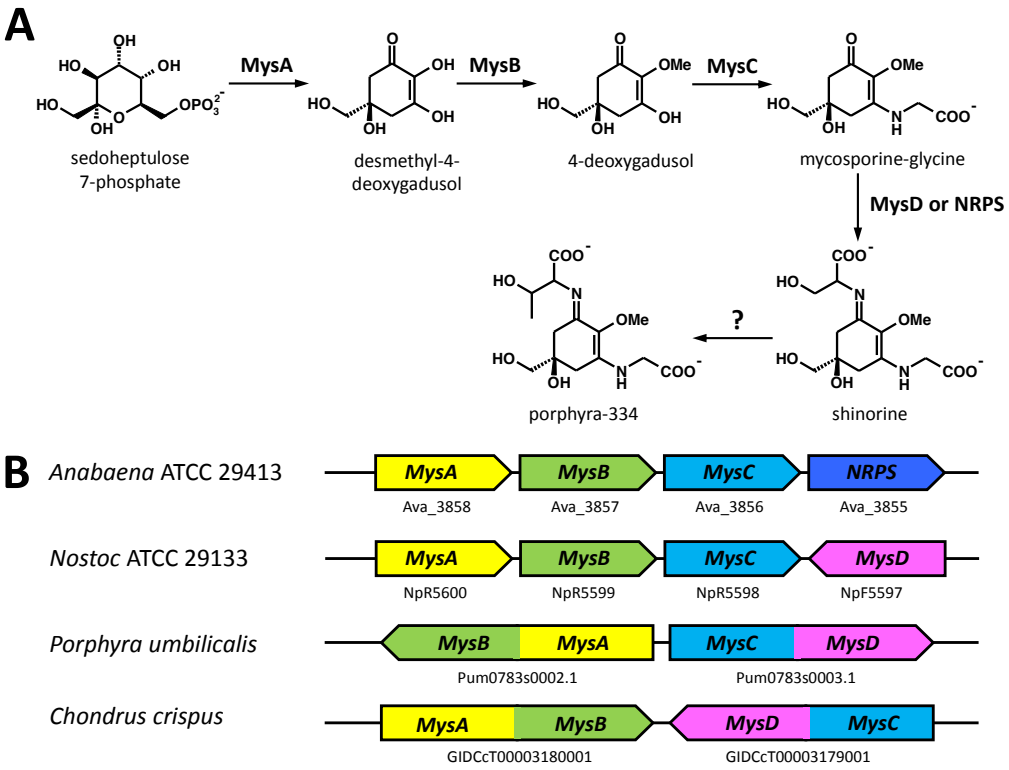

A
MysA
MysB
MysC
MysD or NRPS
?
sedoheptulose 7-phosphate
desmethyl-4-deoxygadusol
4-deoxygadusol
mycosporine-glycine
porphyra-334
shinorine
B
Anabaena ATCC 29413
MysA
MysB
MysC
NRPS
Ava_3858
Ava_3857
Ava_3856
Ava_3855
Nostoc ATCC 29133
MysA
MysB
MysC
MysD
NpR5600
NpR5599
NpR5598
NpF5597
Porphyra umbilicalis
MysB
MysA
MysC
MysD
Pum0783s0002.1
Pum0783s0003.1
Chondrus crispus
MysA
MysB
MysD
MysC
GIDCcT00003180001
GIDCcT00003179001

Figure 4

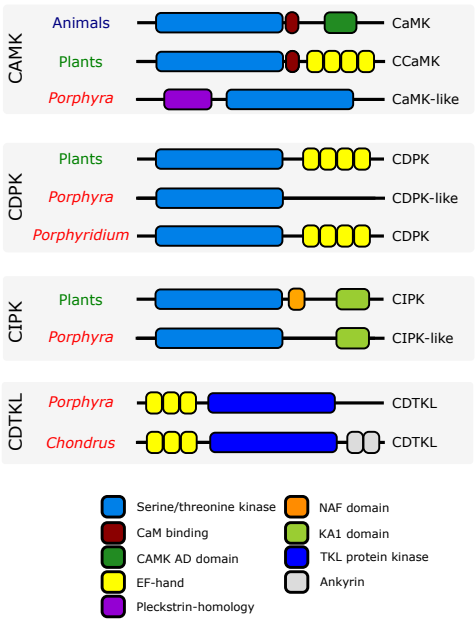

CAMK
Animals
CaMK
Plants
CCaMK
Porphyra
CaMK-like
CDPK
Plants
CDPK
Porphyra
CDPK-like
Porphyridium
CDPK
CIPK
Plants
CIPK
Porphyra
CIPK-like
CDTKL
Porphyra
CDTKL
Chondrus
CDTKL
Serine/threonine kinase
CaM binding
CAMK AD domain
EF-hand
Pleckstrin-homology
NAF domain
KA1 domain
TKL protein kinase
Ankyrin

Figure 5

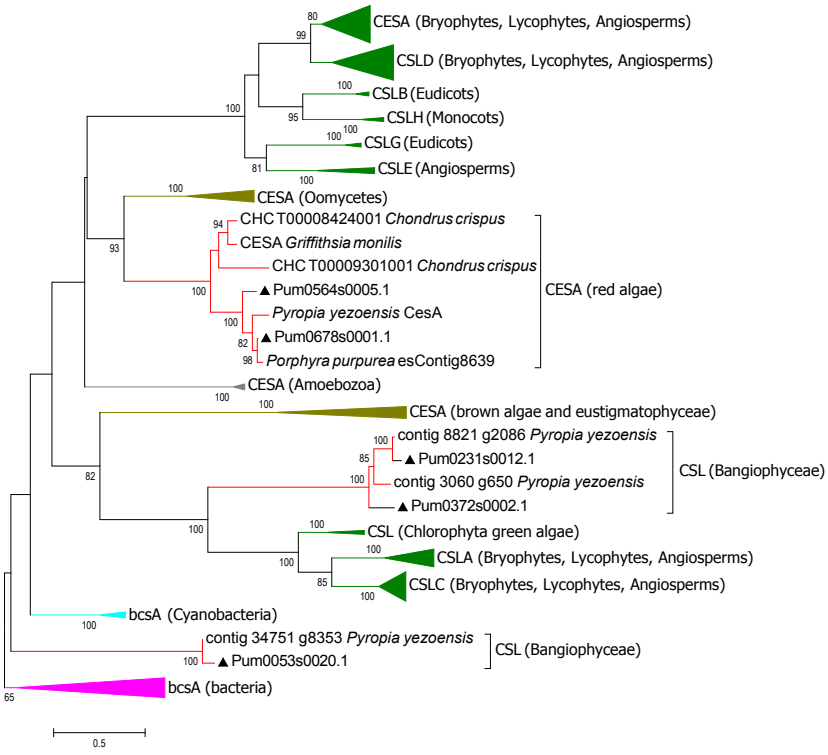
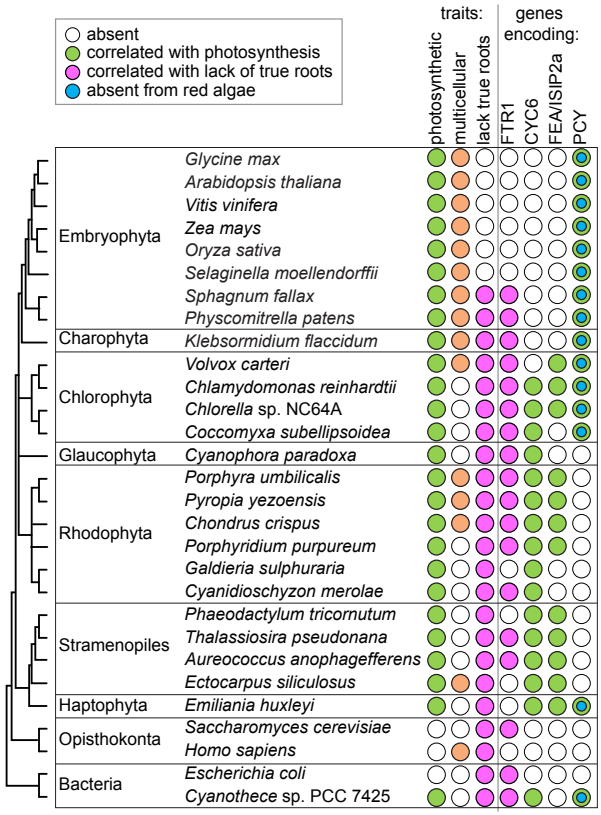

CESA (Bryophytes, Lycophytes, Angiosperms)
CSLD (Bryophytes, Lycophytes, Angiosperms)
CSLB (Eudicots)
CSLH (Monocots)
CSLG (Eudicots)
CSLE (Angiosperms)
CESA (Oomycetes)
CHC T00008424001 Chondrus crispus
CESA Griffithsia monilis
CHC T00009301001 Chondrus crispus
Pum0564s0005.1
Pyropia yezoensis CesA
Pum0678s0001.1
Porphyra purpurea esContig8639
CESA (red algae)
CESA (Amoebozoa)
CESA (brown algae and eustigmatophyceae)
contig 8821 g2086 Pyropia yezoensis
Pum0231s0012.1
contig 3060 g650 Pyropia yezoensis
Pum0372s0002.1
CSL (Bangiophyceae)
CSL (Chlorophyta green algae)
CSLA (Bryophytes, Lycophytes, Angiosperms)
CSLC (Bryophytes, Lycophytes, Angiosperms)
bcsA (Cyanobacteria)
contig 34751 g8353 Pyropia yezoensis
Pum0053s0020.1
CSL (Bangiophyceae)
bcsA (bacteria)
0.5

Figure 6


absent
correlated with photosynthesis
correlated with lack of true roots
absent from red algae
traits:
genes encoding:
photosynthetic
multicellular
lack true roots
FTR1
CYC6
FEA/ISIP2a
PCY
Embryophyta
Glycine max
Arabidopsis thaliana
Vitis vinifera
Zea mays
Oryza sativa
Selaginella moellendorffii
Sphagnum fallax
Physcomitrella patens
Charophyta
Klebsormidium flaccidum
Chlorophyta
Volvox carteri
Chlamydomonas reinhardtii
Chlorella sp. NC64A
Coccomyxa subellipsoidea
Glaucophyta
Cyanophora paradoxa
Rhodophyta
Porphyra umbilicalis
Pyropia yezoensis
Chondrus crispus
Porphyridium purpureum
Galdieria sulphuraria
Cyanidioschyzon merolae
Stramenopiles
Phaeodactylum tricornutum
Thalassiosira pseudonana
Aureococcus anophagefferens
Ectocarpus siliculosus
Haptophyta
Emiliania huxleyi
Opisthokonta
Saccharomyces cerevisiae
Homo sapiens
Bacteria
Escherichia coli
Cyanothece sp. PCC 7425